Null Results in Brief

No Association between Cytochrome P450 and Glutathione *S*-Transferase Gene Polymorphisms and Risk of Colorectal Adenoma: Results from the UK Flexible Sigmoidoscopy Screening Trial

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Introduction

Genetic variation in carcinogen metabolizing enzymes has been proposed as a susceptibility marker for colorectal neoplasia. The cytochrome P450 (CYP) and glutathione *S*-transferase (GST) enzymes metabolize several classes of carcinogen in the human diet and tobacco smoke. Epidemiologic studies that have sought to investigate the relation between variants in *CYP* and *GST* genes and colorectal neoplasia have thus far yielded conflicting results and a consensus regarding their etiologic importance has yet to be reached (1). In light of this, we conducted a case-control study, nested within a large randomized controlled trial, to determine whether functionally characterized variants of the *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, and *GSTM3* genes are associated with risk of colorectal adenoma. In addition, we investigated the interaction between specific dietary components, smoking, genotype, and colorectal adenoma risk.

Materials and Methods

Study Sample. The study included 1,899 Caucasian individuals (1,267 males and 632 females), ages 55 to 64 years, who had undergone screening for polyps in the distal colorectum as part of the UK Flexible Sigmoidoscopy Screening Trial (2). The UK Flexible Sigmoidoscopy Screening Trial is a randomized controlled trial of 368,583 participants from 14 geographic regions, designed to test the efficacy of a once-only flexible sigmoidoscopy in the prevention of colorectal cancer. Individuals were invited to participate via their general practitioner. Of the 40,674 screened, 131 colorectal cancers were detected and excluded from the analysis. Other exclusion criteria included a history of colorectal cancer, adenoma, or inflammatory bowel disease; a severe or terminal disease with life expectancy of <5 years; and a sigmoidoscopy or colonoscopy within the past 2 years or incapability of providing informed consent. In the study presented here, cases were individuals with histologically confirmed adenoma of the distal bowel from three of the study centers (Leeds, Norwich, and Portsmouth). All adenoma cases were asked to provide a blood specimen, of which 94% agreed. Controls were age- and sex-matched individuals with a negative flexible sigmoidoscopy result (no adenomatous or hyperplastic polyps). Overall, blood samples were available for 918 cases and 981 controls.

Assessment of Smoking Habit and Diet. Before screening, participants completed a questionnaire regarding how often 26 selected food items were eaten. The dietary items analyzed in this study comprise components known to interact with the cytochrome P450 and GST enzyme systems and included subtypes of cruciferous vegetables, different types of red and processed meat, and usual meat cooking methods. Participants were assigned as never smokers, former smokers, and current smokers.

Genotyping. Polymorphisms were selected on the basis of whether they lead to functional changes in the translated protein, their prevalence in Caucasian populations, and any previous association with colorectal neoplasia (Table 1). The methods used to discriminate the *GSTM1, GSTT1*, *GSTM3*, and *CYP1A1* alleles have been described elsewhere (3, 4). The *CYP2E1*5B* allele was distinguished by amplification of a 480-bp fragment that carries two linked variants: a C(−1091)T transition recognized by *Pst*I and a G(−1259)C transversion recognized by *Rsa*I. The *CYP2E1*5B* allele is determined by a *Pst*I cut, *Rsa*I noncut. Primer sequences were 5′-ACTGGAAAGGAAAGAGAGGAG-3′ (sense) and 5′-CATTCTGTCTTCTAACTGGCA-3′ (antisense).

Statistical Analysis. Differences in genotype distributions between cases and controls were ascertained by the χ^2 statistic. Risks were calculated as odds ratios with 95% confidence intervals by unconditional logistic regression and were adjusted for age, sex, and sigmoidoscopy center. To test for modification of the association between the dietary variables, smoking, and adenoma risk by genotype, a stratified analysis was conducted by genotype. Potential two-way interactions between genotype and the dietary

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Table 1. List of studied genes and polymorphisms

Gene	Polymorphism	Variant phenotype
CYP1A1	3801_{T-C} (rs4646903)	In 3′ untranslated region, linked with increased *CYP1A1* inducibility.
	2455_{A-G} (rs1048943)	$Ile^{462}Val$ amino acid change, Val enzyme has higher inducibility.
CYP2E1	-1294_{C-G} (rs3813867)	In 5′ untranslated region, predicts higher enzyme levels.
GSTM1	Null	No expression.
GSTT1	Null	No expression.
GSTM3	AGG/- (intron 6 deletion; rs1799735)	Generates recognition site for YY1 transcription factor.

variables were assessed by comparing models with and without the interaction term using the likelihood ratio test. Interactions were considered to be statistically significant at the 1% level.

Results

None of the genotype distributions in the controls differed significantly from those expected under Hardy-Weinberg equilibrium and all were in the range reported previously for Caucasians (ref. 5; Table 2). Carriage of the *CYP1A1*2C* allele was inversely associated with adenoma risk (odds ratio, 0.7; 95% confidence interval, 0.5-0.9). We did not detect any association with alleles of the other genes and colorectal adenoma risk. Subgroup analyses revealed no effect of gender on genotypic risks. Risk estimates did not differ according to genotype and we found no evidence for multiplicative interaction between diet and smoking, genotype, and adenoma (data not shown). Overall results for diet and smoking from the UK Flexible Sigmoidoscopy Screening Trial study will be published elsewhere.

Discussion

This is the largest study, to date, to examine the interaction of diet, smoking, and metabolic gene polymorphisms and colorectal adenoma risk. We detected an inverse association between carriage of the *CYP1A1*2C* allele and risk of colorectal adenoma but no association with the other genotypes. We found no evidence for an interaction between diet, smoking, and genotype in adenoma risk.

The finding that the *CYP1A1*2C* allele was inversely related to colorectal adenoma was unexpected and conflicts with previous studies where a positive association or no association was identified (6-12). Given the large number of genotypes examined in this study, this finding may have arisen by chance.

One of the strengths of this study is a control group known to be free of distal lesions. However, some degree of misclassification may have occurred as adenomas in the proximal colon cannot be detected during sigmoidoscopy.

Although this is the largest study of colorectal adenoma and *CYP/GST* polymorphisms to date, statistical power was compromised in several analyses; we had ~50% power to detect the 30% reduction in risk associated with the *CYP1A1*2C*-carrier genotype. Larger studies are necessary to confirm the modest reduction in colorectal adenoma risk associated with this allele. However, the low prevalence of the *CYP1A1*2C* allele raises questions as to the overall impact this variant may have on colorectal neoplasia risk in the Caucasian population.

In conclusion, we report an overall lack of association between common variants of these xenobiotic metabolism genes and colorectal adenoma risk. Given the bipartite mechanism of carcinogen metabolism, studies should be done with adequate statistical power to assess such variants in a combinatorial manner.

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Table 2. Genotype frequencies in cases and controls and colorectal adenoma risks

Gene	Genotype	Cases (*N*)	Controls (*N*)	Odds ratio* (95% confidence interval)
CYP1A1	*1/*1	864 (94.3%)	895 (91.7%)	1.0†
	*1/*2C	52 (5.7%)	80 (8.2%)	0.7 (0.5-0.9)
	*2C/*2C	0 (0%)	1 (0.1%)	—
	*2C carrier‡	52 (5.7%)	81 (8.3%)	0.7 (0.5-0.9)
CYP1A1	*1/*1	745 (84.1%)	738 (83.2%)	1.0†
	*1/*2A	138 (15.6%)	142 (16.0%)	1.0 (0.8-1.2)
	*2A/*2A	3 (0.3%)	7 (0.8%)	0.4 (0.1-1.7)
	*2A carrier‡	141 (15.9%)	149 (16.8%)	0.9 (0.7-1.2)
CYP2E1	*1/*1	865 (95.0%)	918 (94.5%)	1.0†
	*1/*5B	46 (5.0%)	53 (5.5%)	0.9 (0.6-1.4)
	*5B/*5B	0 (0%)	0 (0%)	—
GSTM1	Null (0/0)	556 (64.7%)	552 (62.4%)	1.0†
	A/A or *A/0*	150 (17.5%)	179 (20.2%)	0.8 (0.7-1.1)
	B/B or *B/0*	131 (15.3%)	135 (15.3%)	1.0 (0.7-1.3)
	A/B	22 (2.5%)	19 (2.1%)	1.2 (0.6-2.2)
GSTM1	Non-null	303 (35.3%)	333 (37.6%)	1.0†
	Null	556 (64.7%)	552 (62.4%)	1.1 (0.9-1.4)
GSTM3	*A/A*	649 (70.9%)	692 (70.8%)	1.0†
	A/B	246 (26.9%)	255 (26.1%)	1.0 (0.8-1.3)
	B/B	21 (2.2%)	30 (3.1%)	0.8 (0.4-1.3)
	*B carrier‡	267 (29.1%)	285 (29.2%)	1.0 (0.8-1.2)
GSTT1	Non-null	644 (83.9%)	672 (82.6%)	1.0†
	Null	124 (16.1%)	142 (17.4%)	0.9 (0.7-1.2)

NOTE: Absolute numbers (*N*) differ slightly due to differences in efficacy of genotyping protocols.
*Odds ratios were adjusted for age, sex, and screening center.
†Reference category.
‡Because of the low frequency of *CYP1A1*2A* and *2C homozygotes, a ''carrier'' category was created that included *CYP1A1*1/*2A* and *CYP1A1*2A/*2A* as a *CYP1A1*2A* carrier genotype, *CYP1A1*1/*2C* and *CYP1A1*2C*/2C* as a *CYP1A1*2C* carrier genotype, and *GSTM3*A*B* and *GSTM3*B*B* as a *GSTM3*B* carrier genotype.

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